

activated when the natural ligand PDGF binds simultaneously to the ectodomains of two receptors, resulting in spatial proximity and subsequent cross-activation of the cytosolic kinase domains¹). The receptor can also be activated independently of the natural ligand by binding of the small E5 oncoprotein from bovine papillomavirus²). E5 is a small membrane protein of only 44 amino acids and it is thought to manipulate the function of the receptor by specific helix-helix-contacts to the transmembrane of the receptor which then result in sustained receptor activation and cell transformation.

To elucidate the helix-helix-interactions in receptor complex, we investigate the structure and membrane alignment of both proteins, first for each protein and later in the heterotetrameric four-helix-bundle. For the structural analysis we combined circular dichroism (CD) spectroscopy and liquid-state NMR to determine the secondary structure. Oriented CD and solid-state NMR were used to resolve the orientation of both proteins in their native environment. Therefore the E5 protein and the transmembrane domain of the receptor (PDGFRTMD) were ¹⁵N-isotope labelled by bacterial protein expression and reconstituted in detergent micelles and in oriented lipid bilayers. By CD and liquid-state NMR we found that both proteins are predominantly α -helical in detergent micelles and in lipid bilayers^{3,4}). Furthermore, high resolution NMR measurements showed that PDGFRTMD forms a left-handed coiled-coil structure. A complementary OCD and solid-state NMR analysis of E5 and PDGFRTMD reconstituted in different lipid bilayers showed that the orientation of both proteins depends on the bilayer thickness, where both proteins were more tilted in thin membranes and less tilted in thick membranes.

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Structure and Dynamics of Membrane-Associated Hepatitis C Virus Protein P7 by NMR Spectroscopy

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P7 is a 63-residue viroporin encoded by human hepatitis C virus (HCV). It has ion channel activity and plays essential roles in viral proliferation. The NMR results show that the protein consists of two transmembrane spanning regions connected by a cytoplasmic loop. The solution NMR structural data that has been acquired includes: hydrogen/deuterium exchange, paramagnetic relaxation enhancement, residual dipolar couplings, and bicelle 'q-titration.' These data demonstrate that the protein has a range of dynamic properties, and interestingly two segments in each of the membrane spanning helices display local motions, possibly related to function. Oriented Sample (OS) solid-state NMR spectra of p7 in aligned phospholipid bilayers provided the tilt angles of the helical segments, 25° and 10° to the membrane normal. Recent magic angle spinning (MAS) solid-state NMR data of p7 in liposomes are being used to determine the three-dimensional structure. It has been shown that known channel-blocking compounds inhibit the ion channel activity. This and the fact that knockout models of this protein inhibit infectivity of the virus indicate it may be an ideal target for future drugs. There are six major genotypes and over 100 relevant subtypes. Our current studies aim to investigate the structure and dynamic differences among the genotypes. In addition to studying different genotypes, specific mutations to the HCV J4 genotype were made in order to modulate the dynamics of the protein and obtain additional secondary structural information. These studies have the potential to identify the reasons why different genotypes have varying activity and drug binding affinities.

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Solid-State NMR Studies on the Membrane Bound Acylated Peptide Hormone Ghrelin

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Ghrelin is a 28-amino acid peptide, which is known to play a key role in the regulation of food intake and body weight. Ghrelin is the only known hormone that carries an octanoyl lipid modification at residue Ser 3. Here, we have studied the structure and dynamics of the molecule bound to DMPC/DMPS (80/20) membranes by solid-state NMR spectroscopy. We have synthesized several Ghrelin peptides with varying ¹³C/¹⁵N containing amino acids covering 10 out of the 28 residues in the sequence. ¹³C NMR spectra recorded under magic-angle spinning conditions provide backbone torsion angles for the membrane-associated peptide. Further, the molecular dynamics of Ghrelin was studied using motional averaged dipolar coupling measurements. Overall, the data indicates a rather flexible peptide conformation on the mem-

brane surface. We have further studied the conformation and membrane insertion of the octanoyl lipid modification of Ghrelin using 2H NMR. Ghrelin binding leads to a small alteration in the order parameter profile of the host membrane. Further, we have also measured the 2H NMR spectrum of a Ghrelin molecule with a deuterated octanoyl chain. This spectrum showed clear indications of a membrane inserted lipid chain, however, the chain order parameters of the lipid modification are smaller translating into a somewhat shorter chain length compared to the host membrane. Experimentally, we determined a membrane binding energy of ~40 kJ/mol for Ghrelin, which cannot solely be explained by the insertion of hydrophobic amino acids and the octanoyl chain. In addition, electrostatic forces play a role arising from the 7 positively charged residues in the peptide sequence. In the course of action, Ghrelin binds to the GHS receptor, so membrane binding might be an important step increasing the Ghrelin concentration at the membrane prior to binding to its receptor.

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Towards the Backbone Structure Determination of the Membrane Protein, LspA, from *Mycobacterium Tuberculosis* using Solid State NMR

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Lipoprotein Signal Peptidase A (LspA) is an essential enzyme in one of the three distinct steps in lipoprotein maturation. After targeting, the signal peptide of a prolipoprotein, the peptide is cleaved by LspA to result in the mature lipoprotein.

In *Mycobacterium tuberculosis* (*Mtb*), LspA is encoded by the gene RV1539 resulting in a 202 amino acid residue peptide with a molecular weight is 21 kDa that is predicted to possess four transmembrane helices. It has been recognized *in vivo* that LspA is an essential protein for *Mtb* virulence and that it may also contribute to antibiotic resistance, as a result LspA has been identified as a potential drug target for the treatment of tuberculosis. Globomycin, a cyclic-peptide is known to inhibit LspA in a non-competitive manner.

Solid-state NMR uniformly aligned samples is being used to determine the structure of the protein backbone. Anisotropic ¹⁵N-¹H dipolar couplings and ¹⁵N chemical shifts are obtained from separated local field NMR spectra of the protein in aligned bilayer preparations. The resulting orientational restraints can be used to generate a high resolution structure as well as a determination of how the helices are oriented with respect to their environment.

In this study, LspA is reconstituted into synthetic lipids (DMPC/DMPG and POPC/POPG) to mimic the membrane environment and it is aligned uniaxially either mechanically (using glass slides) or magnetically (using bicelles).

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Initial Structural Studies of Mtb Membrane Protein "CrgA" in Membrane Mimetic Environment

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Here we present initial NMR Spectroscopic studies of CrgA, one of the important putative inner membrane proteins in *Mycobacterium tuberculosis* (*Mtb*). CrgA helps in the cell division of *Mtb* contains 2 transmembrane helices (TM), 93 amino acid and is an important drug target. Our studies show that, CrgA interacts with FtsZ (involves in Zring formation during cell division) and Rv0008c (another putative membrane protein) in *Mtb* during the cell division. For NMR structural studies ¹⁵N, ¹³C uniform and amino acid specific isotope labeled CrgA reconstituted in POPC-POPG (4:1 mol/mol) liposomes was used for planar lipid bilayer oriented on glass slides and Magic Angle Spinning (MAS) correlation spectroscopy. The key behind these studies is to determine CrgA structure in membrane mimetic lipid bilayer environment. For oriented glass slide samples, 2D PISEMA experiments were performed which correlates ¹H-¹⁵N dipolar coupling with the ¹⁵N chemical shift from the backbone resonances of ¹⁵N labeled protein in lipid bilayer. Each measured frequency reflects the orientation of the peptide plane of the protein w.r.t to the bilayer normal. Similarly the tilt angles of various parts of the protein (TM, N and C terminal) can also be obtained which is very useful for the structure calculation process. Moreover MAS experiments can measure distances between side chains of various residues. Altogether the aim of this research is to characterize the 3D backbone as well as the side chain structures of CrgA in native membrane like environment.